Thyroid status and endothelium-dependent vasodilation in skeletal muscle

R. M. McAllister,1 I. Albarracin,1 J. L. Jasperse,2 and E. M. Price3

Departments of Anatomy and Physiology and Kinesiology, Kansas State University, Manhattan, Kansas; Department of Sports Medicine, Pepperdine University, Malibu, California; and Department of Veterinary Biomedical Sciences and Dalton Cardiovascular Research Center, University of Missouri, Columbia, Missouri

Submitted 3 February 2003; accepted in final form 7 September 2004

McAllister, R. M., I. Albarracin, J. L. Jasperse, and E. M. Price. Thyroid status and endothelium-dependent vasodilation in skeletal muscle. Am J Physiol Regul Integr Comp Physiol 288: R284–R291, 2005.—Cardiovascular dysfunction is characteristic of both hypothyroidism and hyperthyroidism. Endothelium-dependent dilation of conductance vessels is impaired in hypothyroidism but augmented in hyperthyroidism. We hypothesized that these alterations in dilation extend into the resistance vasculature of skeletal muscle. To test this hypothesis, rats were made hypothyroid with propylthiouracil (Hypo; n = 13) or hyperthyroid with triiodothyronine (Hyper; n = 9) over 3–4 mo. Compared with euthyroid controls (Eut; n = 14), Hypo rats were characterized by reduced skeletal muscle oxidative capacity and blunted growth; Hyper rats exhibited increased muscle oxidative capacity and left ventricular hypertrophy (P < 0.05 for all effects). Vasodilation to the endothelium-dependent agent acetylcholine (~2 × 10–4 M) in skeletal muscle was determined in situ. Conductance in certain muscles increased from control [e.g., soleus: 0.98 ± 0.15 (Eut), 0.79 ± 0.14 (Hypo), and 1.06 ± 0.24 ml⋅min−1⋅100 g−1⋅mmHg−1 (Hyper); not significant among groups] to acetylcholine [1.91 ± 0.21 (Eut), 2.28 ± 0.26 (Hypo), and 2.15 ± 0.33 ml⋅min−1⋅100 g−1⋅mmHg−1 (Hyper); P < 0.05 vs. control values for all groups] but did not differ among groups. Expression of mRNA for the endothelial isofrom of nitric oxide synthase in resistance vessels isolated from various muscles was similarly unchanged with alterations in thyroid status [e.g., soleus 1A arterioles: 33.15 ± 0.58 (Eut), 32.73 ± 0.27 (Hypo), and 32.80 ± 0.54 (Hyper) cycles at threshold; not significant]. These data suggest that endothelium-dependent dilation of resistance vasculature in skeletal muscle is unchanged in both hypothyroidism and hyperthyroidism. These data also emphasize the importance of examining resistance vasculature to improve understanding of effects of chronic disease on integrated cardiovascular function.

hypothyroidism; hyperthyroidism; acetylcholine; muscle fiber type; nitric oxide synthase

Method

Animal treatments. Male Sprague-Dawley rats (Charles River) were housed three per cage in a room with controlled temperature (20–21°C) and light (12:12-h light-dark cycle). Rats initially weighed 150–175 g and were randomly assigned to one of three groups: euthyroid control (Eut), hypothyroid (Hypo), and hyperthyroid (Hyper). Rats assigned to the Hypo group were rendered hypothyroid with the agent propylthiouracil in their drinking water (0.04 g/100 ml) over 3–4 mo, as previously reported (3, 11, 13). Rats assigned to the Hyper group were made hyperthyroid via intraperitoneal injections of triiodothyronine (300 μg/kg body wt) on alternate days over 3–4 mo, as previously reported (12–14). Rats were allowed food and water ad libitum. During the final ~1 mo of the treatment period, Eut rats were slightly food restricted (~90% of normal bulk food intake) for the purpose of matching their body weights with those of Hypo and Hyper rats. All treatments were approved by the Institutional Animal Care and Use Committee of Kansas State University.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Treatment efficacy. Effectiveness of propylthiouracil and triiodothyronine treatments in rendering rats hypothyroid, respectively, was assessed by determining citrate synthase activity in several skeletal muscles: the slow oxidative (SO)-type soleus muscle, the fast oxidative-glycolytic (FOG)-type red section of vastus lateralis muscle, and the fast glycolytic (FG)-type white section of vastus lateralis muscle (1). In the eNOS expression series, because of sampling constraints, citrate synthase activity was determined in the vastus intermedius muscle, which also consists primarily of SO fibers (1).

Activity of citrate synthase, a marker enzyme for oxidative capacity, was determined spectrophotometrically (Shimadzu) according to the method of Srere (19). In addition, left ventricular weight-to-body weight ratio was determined to assess treatment efficacy.

Isolated perfused rat hindlimb preparation. Use of the perfused rat hindlimb preparation for determination of vascular function in skeletal muscle has been described in detail previously (9). Briefly, the left hindlimb was perfused via the femoral artery with oxygenated (95% O2-5% CO2 gas mixture) perfusate containing bovine erythrocytes (hematocrit ~40%), bovine serum albumin (4 g/100 ml), bovine insulin (100 μU/ml), glucose (5.0 mM), and pyruvate (0.15 mM). A constant-flow (total inflow to the hindlimb of 8–9 ml/min), variable-pressure approach was utilized.

Experimental design. During experiments of the perfused rat hindlimb series, perfusion pressure was monitored continuously (Digi-Med blood pressure analyzer), and regional flows (radiolabeled microspheres; see below) were determined at three time points: control, at peak effect of the endothelium-dependent vasodilator acetylcholine, and at peak effect of the endothelium-independent vasodilator sodium nitroprusside. An acetylcholine stock solution of 1.10 × 10−2 M was infused at a rate of 0.15 ml/min, representing 1–2% of total inflow to the hindlimb and establishing a femoral arterial acetylcholine concentration of 1–2 × 10−4 M. A sodium nitroprusside stock solution of 2.68 × 10−2 M was infused at 0.15 ml/min, establishing a femoral arterial nitroprusside concentration of 4–5 × 10−4 M.

Determination of regional flows. Muscle- and/or tissue-specific flows were determined at the time points specified above, using radiolabeled microspheres (46Sc, 85Sr, 113Sn, or 141Ce; 15-μm diameter; New England Nuclear). After an experiment was completed, all muscles and other tissues of the left hindlimb were dissected, weighed, and counted in a gamma counter (Packard). Flows were calculated with the following formula: Flow (muscle) = [counts/min (muscle)/counts/min (total)] × flow (total), in which counts/min (total) refers to the sum of counts/min for all hindlimb muscles and/or tissues and flow (total) refers to total inflow to the hindlimb.

Determination of eNOS expression in resistance vessels. In experiments of the eNOS expression series, resistance vessels from the SO-type soleus (1A arterioles) and FOG-type gastrocnemius muscles (red section; 2A arterioles), as well as the FG-type white section of gastrocnemius muscle (2A arterioles), were dissected and frozen at −80°C until analysis. As described previously (7), a 1A arteriole was defined as the first intramuscular resistance vessel; daughters of this 1A arteriole were defined as 2A arterioles.

Resistance vessels were lysed with a buffer consisting of 100 mM Tris-HCl, 500 mM LiCl, 10 mM EDTA, 1.0 ml/100 ml lithium disulphide, and 5 mM dithiothreitol, pH 7.5. We isolated mRNA from this lysate using magnetic beads with oligo(dT), as described previously (22). First-strand synthesis (i.e., cDNA synthesis) was performed on isolated mRNA with reverse transcriptase (200 U/ml) and oligo(dT).

cDNA was amplified via polymerase chain reaction (PCR). Primers for DNA polymerase to amplify eNOS cDNA were 5′-AGG CAT CAC CAG GAA GAA GA-3′ (forward) and 5′-GCC CAG TCT CAG AGC CAT AC-3′ (reverse; Ref. 17). GAPDH cDNA was amplified with the primers 5′-ACT CTA CCC ACG GCA AGT TC-3′ (forward) and 5′-TAC GCA GCA CCA GCA TCA CC-3′ (reverse; Ref. 17). These primers yield products <150 bp in size, a requirement of mRNA quantification with SYBR Green real-time PCR technology (17). Real-time PCR cycles (40×) consisted of 30 s at 95°C (denaturing), 60 s at 60°C (annealing), and 60 s at 75°C (extension; Ref. 17). The reaction mixture included a master mixture (dNTPs, DNA polymerase, SYBR Green), MgCl2 (2.5 mM), primers (100 nM), and either 5.0 μl of cDNA (eNOS) or 2.0 μl of cDNA (GAPDH; Ref. 17). Samples were analyzed in duplicate. PCR products were confirmed by melt curves and by 1.5 g/100 ml agarose gels with appropriate controls (e.g., no reverse transcriptase). eNOS expression was normalized to GAPDH expression.

Fig. 1. Citrate synthase activity in selected hindlimb skeletal muscles. VL, vastus intermedius muscle; VL (red), red section of vastus lateralis muscle; VL (white), white section of vastus lateralis muscle. Values are means ± SE; n = 14, 13, and 9 for soleus of euthyroid (open bars), hypothyroid (hatched bars), and hyperthyroid (stippled bars) preparations, respectively; n = 5, 4, and 5 for VL (red) and VL (white) of euthyroid, hypothyroid, and hyperthyroid preparations, respectively; n = 19, 17, and 14 for VL (red) and VL (white) of euthyroid, hypothyroid, and hyperthyroid preparations, respectively. aDifferent from euthyroid, P < 0.05. †Different from hypothyroid, P < 0.05.
These muscles are classified as high oxidative because they are anterior, tibialis posterior, and peroneal muscles or muscle sections. The low oxidative muscle group included the soleus, plantaris, red gastrocnemius, red tibialis longus, and flexor halicus longus muscles. These muscles or muscle sections were composed of 50% SO and/or FOG fibers (1). The low oxidative muscle group included the white gastrocnemius, mixed gastrocnemius, white tibialis anterior, extensor digitorum longus, flexor digitorum longus, and flexor hallucis longus muscles. These muscles or muscle sections were composed of <50% SO and/or FOG fibers (1). Fat, tibia and fibula, and foot composed the other tissue groups. Data for muscles and tissues of the upper hindlimb were necessarily included in all flow calculations but are not presented in RESULTS, because variable femoral arterial catheter placement prevented reliable flow determinations in this portion of the hindlimb.

For a given muscle or tissue or grouping, conductance data were analyzed using two-way ANOVA with repeated measures across time points (i.e., control, acetylcholine, nitroprusside; Ref. 20). Data for citrate synthase activity, weights, perfusion variables, and eNOS expression were analyzed with one-way ANOVA (20). The Tukey's test was used for post hoc analysis (20). P < 0.05 was considered significant for all analyses.

RESULTS

Treatment efficacy. Treatment of rats with propylthiouracil and triiodothyronine induced hypo- and hyperthyroidism, respectively. Citrate synthase activity was reduced in Hypo and increased in Hyper rats, relative to Eut rats, in the high oxidative soleus, vastus intermedius, and vastus lateralis (red section) muscles (Fig. 1). In addition, Hypo rats failed to gain body weight at a normal rate, as reflected by lower final body weight in the perfused hindlimb series (Table 1). In the eNOS expression series, Hyper rats were also of lower body weight (Eut: 430 ± 15 g, Hypo: 232 ± 13 g, Hyper: 378 ± 19 g; P < 0.05, Hypo vs. Eut, Hyper). Hyper rats exhibited left ventricular hypertrophy, as reflected by a higher left ventricular weight-to-body weight ratio (Eut: 1.94 ± 0.03 mg/g, Hypo: 1.96 ± 0.05 mg/g, Hyper: 2.65 ± 0.10 mg/g; P < 0.05, Hyper vs. Eut, Hypo).

Perfusion conditions. Hindlimbs of Eut, Hypo, and Hyper rats were provided with similar cardiovascular support, as indicated by similar values for perfusate hematocrit and total inflow (Table 1). Perfusion pressure under control conditions was greater in Hypo and Hyper than in Eut rats (Table 1).

Vasodilatory responses to acetylcholine and nitroprusside. Femoral arterial concentrations of acetylcholine established were 1.85 ± 0.01 × 10⁻⁴ M, 1.92 ± 0.02 × 10⁻⁴ M, and 1.86 ± 0.02 × 10⁻⁴ M for Eut, Hypo, and Hyper rats, respectively, and resulted in decreases in perfusion pressure (Table 1). Magnitude of the acetylcholine-induced decrease in perfusion pressure was greater (P < 0.05) in both Hypo and Hyper (Hypo: 21 ± 3 mmHg, Hyper: 26 ± 3 mmHg) than in Eut rats (9 ± 1 mmHg). Concentrations of nitroprusside established were 4.53 ± 0.03 × 10⁻⁴ M, 4.74 ± 0.06 × 10⁻⁴ M, and 4.53 ± 0.04 × 10⁻⁴ M for Eut, Hypo, and Hyper rats, respectively, and also resulted in decreases in perfusion pressure (Table 1). Nitroprusside-induced decreases in perfusion pressure were similar among groups (Eut: 42 ± 9 mmHg, Hypo: 66 ± 13 mmHg, Hyper: 48 ± 16; not significant).

---

Table 1. Weights and perfusion conditions for perfused hindlimb series

<table>
<thead>
<tr>
<th></th>
<th>Euthyroid</th>
<th>Hypothyroid</th>
<th>Hyperthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weights</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body, g</td>
<td>512±14</td>
<td>268±5†‡</td>
<td>493±9</td>
</tr>
<tr>
<td>Hindlimb, g</td>
<td>33.3±1.0</td>
<td>19.9±0.5†‡</td>
<td>31.5±0.7</td>
</tr>
<tr>
<td><strong>Perfusion conditions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>38±1</td>
<td>38±1</td>
<td>38±1</td>
</tr>
<tr>
<td>Total inflow, ml/min</td>
<td>8.9±0.1</td>
<td>8.6±0.1†‡</td>
<td>8.9±0.1</td>
</tr>
<tr>
<td>Perfusion pressure (control), mmHg</td>
<td>81±2</td>
<td>102±4*</td>
<td>100±4*</td>
</tr>
<tr>
<td>Perfusion pressure (pre-/postagent), mmHg</td>
<td>83±4</td>
<td>102±7†</td>
<td>84±4</td>
</tr>
<tr>
<td>Preacetylcholine</td>
<td>84±2</td>
<td>111±5*†</td>
<td>102±4*</td>
</tr>
<tr>
<td>Postacetylcholine</td>
<td>76±1</td>
<td>90±3†‡</td>
<td>76±2</td>
</tr>
<tr>
<td>Pre nitroprusside</td>
<td>125±11</td>
<td>168±15</td>
<td>131±17</td>
</tr>
<tr>
<td>Post nitroprusside</td>
<td>83±4</td>
<td>102±7†</td>
<td>84±4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 14, 13, and 9 for euthyroid, hypothyroid, and hyperthyroid preparations, respectively. *Different from euthyroid, P < 0.05; †different from hypothyroid, P < 0.05.

Data analysis. All data are presented as means ± SE. Vascular conductance was used as an expression of vasomotor function because of its linear relationship with flow (8). Conductance was calculated as the quotient of flow (ml·min⁻¹·100 g⁻¹) and perfusion pressure (mmHg). Conductance data for individual muscles and tissues were analyzed; in addition, muscles and other tissues were combined into groups for analyses. These groups included high oxidative muscle, low oxidative muscle, and other tissues. The high oxidative muscle group included the soleus, plantaris, red gastrocnemius, red tibialis anterior, tibialis posterior, and peroneal muscles or muscle sections. These muscles are classified as high oxidative because they are composed of ≥50% SO and/or FOG fibers (1). The low oxidative muscle group included the white gastrocnemius, mixed gastrocnemius, white tibialis anterior, extensor digitorum longus, flexor digitorum longus, and flexor hallucis longus muscles. These muscles or muscle sections are composed of <50% SO and/or FOG fibers (1). Fat, tibia and fibula, and foot composed the other tissue groups. Data for muscles and tissues of the upper hindlimb were necessarily included in all flow calculations but are not presented in RESULTS, because variable femoral arterial catheter placement prevented reliable flow determinations in this portion of the hindlimb.
Conductance under control conditions and at peak effect of acetylcholine and nitroprusside is presented for the soleus muscle and the red and white sections of the gastrocnemius muscle in Figs. 2–4, respectively. The high oxidative soleus (Fig. 2) and red gastrocnemius muscles (Fig. 3) generally exhibited vasodilation in response to either acetylcholine or nitroprusside, reflected by increases in conductance. In contrast, the low oxidative white gastrocnemius muscle section (Fig. 4) did not exhibit changes in conductance with either vasodilator in any group. Of these muscles, there were effects of thyroid status only in the white gastrocnemius, where conductance was greater in Hyper rats than in either Eut or Hypo rats during nitroprusside administration. Conductance data for remaining muscles or tissues of the lower hindlimb are presented in Table 2. High oxidative muscles, considered as a group, exhibited vasodilation in response to acetylcholine in all

Fig. 3. Conductance in red section of gastrocnemius muscle, composed primarily of fast oxidative/glycolytic fibers. Values are means ± SE; n = 14, 13, and 9 for euthyroid (open bars), hypothyroid (hatched bars), and hyperthyroid (stippled bars) preparations, respectively. *Different from control within same group, P < 0.05.

Fig. 4. Conductance in white section of gastrocnemius muscle, composed primarily of fast glycolytic fibers. Values are means ± SE; n = 14, 13, and 9 for euthyroid (open bars), hypothyroid (hatched bars), and hyperthyroid (stippled bars) preparations, respectively. †Different from hyperthyroid within same condition, P < 0.05.
groups (Fig. 5); however, the low oxidative muscle groupings only showed acetylcholine-induced vasodilation in Hypo and Hyper rats (Fig. 6). Data for Hypo and Hyper rats were, however, not significantly different from Eut rat data in the response of low oxidative muscle to acetylcholine. There were few changes in conductance with either vasodilator in other tissues, but Hypo rats generally exhibited greater conductance in this grouping than either Eut or Hyper rats (Fig. 7, Table 2).

DISCUSSION

The chief new finding of this study was that endothelium-dependent dilation in skeletal muscle vasculature was normal in both the hypo- and hyperthyroid states. This finding improves our understanding of cardiovascular function with alterations in thyroid status and has implications for mechanisms underlying exercise intolerance in hypo- and hyperthyroidism.

Experimental considerations. Our rat models of hypo- and hyperthyroidism were validated by several indicators of treatment efficacy. Hypo rats exhibited reduced skeletal muscle oxidative capacity, a hallmark of the hypothyroid state reported by us (3, 11, 13) and others (e.g., Ref. 5), and also demonstrated blunted growth. Hyper rats exhibited increased muscle oxidative capacity, as shown previously by our laboratory (12–14) and by others (e.g., 5), as well as left ventricular hypertrophy.

Use of the isolated perfused rat hindlimb preparation allowed determination of muscle-specific flow responses to endothelium-dependent and -independent agents. A vasodilatory response in this preparation, reflected by an increase in conductance, represents the integrated response of conductance vessels (e.g., femoral artery) and resistance vessels within individual skeletal muscles. This experimental approach represents an improvement over determination of dilatory responses in a larger, conductance-type vessel (i.e., abdominal aorta), as done previously by our laboratory (3, 12, 13) and by others (18), since most resistance to blood flow resides in the resistance vasculature.

Endothelium-dependent vasodilatory responses: hypothyroidism. Contrary to our hypothesis, skeletal muscles of Hypo rats did not exhibit impaired vasodilatory responses to acetylcholine. Interestingly, vasodilation to acetylcholine occurred in several individual muscles of Hypo rats, but not in Eut rats, including the high oxidative red portions of gastrocnemius and tibialis anterior, tibialis posterior, and peroneals, as well as the low oxidative mixed gastrocnemius, extensor digitorum longus, flexor digitorum longus, and flexor halicus longus muscles (Fig. 3 and Table 2). Eut-Hypo differences were not, however, statistically significant for any of these muscles. Collectively, slightly greater endothelium-dependent vasodilation in these muscles of Hypo rats, but not Eut rats, may account for the larger decrease in perfusion pressure with acetylcholine administration in Hypo compared with Eut rats (Table 1). These findings indicate that endothelium-dependent vasodilation in skeletal muscle is not impaired in the hypothyroid state, contrary to our hypothesis.

Endothelium-dependent vasodilatory responses: hyperthyroidism. Hyper rats also exhibited normal responses to acetylcholine. The peroneal muscle grouping and mixed gastrocnemius muscle section exhibited significant vasodilation in response to acetylcholine administration, responses that did not occur in Eut rats. Eut-Hyper differences were not, however, statistically significant for either of these muscles. Similar to
Fig. 5. Conductance in high oxidative muscle grouping. Values are means ± SE; \( n = 14, 13, \) and 9 for euthyroid (open bars), hypothyroid (hatched bars), and hyperthyroid (stippled bars) preparations, respectively. See METHODS for details of high oxidative muscle grouping. *Different from control within same group, \( P < 0.05 \).

Fig. 6. Conductance in low oxidative muscle grouping. Values are means ± SE; \( n = 14, 13, \) and 9 for euthyroid (open bars), hypothyroid (hatched bars), and hyperthyroid (stippled bars) preparations, respectively. See METHODS for details of low oxidative muscle grouping. *Different from control within same group, \( P < 0.05 \).

Fig. 7. Conductance in other tissues grouping. Values are means ± SE; \( n = 14, 13, \) and 9 for euthyroid (open bars), hypothyroid (hatched bars), and hyperthyroid (stippled bars) preparations, respectively. See METHODS for details of other tissues grouping. *Different from control within same group, \( P < 0.05 \). †Different from hypothyroid within same condition, \( P < 0.05 \).
Hypo rats, Hyper rats exhibited a larger decrease in perfusion pressure than Eut (Table 1), which may have been accounted for by slightly greater endothelium-dependent vasodilation in these two muscles or muscle sections of Hyper rats. Importantly, the mixed gastrocnemius muscle section represents a sizeable fraction of total muscle mass in the rat hindlimb (1). It appears that, again contrary to our hypothesis, endothelium-dependent vasodilation is not augmented in skeletal muscle in hyperthyroidism.

Thyroid status and eNOS expression. Because nitric oxide has been reported to be an important mediator of endothelium-dependent vasodilation in skeletal muscle (7, 9), we determined eNOS mRNA expression in resistance vessels of selected hindlimb muscles. Resistance vessels were harvested from selected muscles consisting primarily of either SO, FOG, or FG fibers. Consistent with vasodilatory data obtained in the perfused hindlimb series (Figs. 2–4), eNOS expression was not altered by thyroid status in either the soleus or gastrocnemius (red and white sections) muscles or muscle sections. The resistance vessels examined represent important sites of vascular control in the skeletal muscle circulation and would contribute to responses to acetylcholine observed in situ. The unchanged eNOS expression in these resistance vessels likely contributed to the lack of alteration in responses to acetylcholine in situ. It appears likely that nitric oxide degradation also did not vary with thyroid status because normal responses to acetylcholine suggest that nitric oxide bioavailability was not changed in either Hypo or Hyper preparations.

Effects of thyroid disease on conductance in other tissues. Tissues other than skeletal muscle exhibited altered vascular control in hypothyroidism. The foot exhibited greater conductance in Hypo than in either Hyper or Eut rats (Table 2, Fig. 7). Greater conductance in peripheral locations such as the foot, if present under in vivo conditions, may contribute to lower body temperature in hypothyroidism (cf Ref. 10) by increasing heat loss via the cutaneous circulation.

Perspectives

Skeletal muscle blood flows during exercise, which are elevated dramatically over resting levels (6), are markedly modulated by thyroid disease. Poor cardiac function may be primarily responsible for markedly blunted muscle blood flows during exercise in hypothyroidism (11), given the normal dilation of skeletal muscle vasculature observed in the present study. Cardiac dysfunction is a hallmark of hypothyroidism (cf Ref. 15). In hyperthyroidism, augmented cardiac function (cf Ref. 15) may be responsible for improved perfusion of muscle during exercise (4, 14), because we did not observe enhanced endothelium-dependent vasodilation in the hyperthyroid state.

ACKNOWLEDGMENTS

The technical assistance of Emily Buhr, Molly Edmonds, Kasee Hildenbrand, Trisha Richards, and Mike Zbreski is gratefully acknowledged. Also acknowledged are the cooperation of the staffs at Alta Vista (KS) Locker, Burlingame (KS) Locker and Meat Market, and Clay Center (KS) Locker, who graciously provided bovine blood, and the helpful advice of Miles Tanner concerning real time PCR.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-57226, American Heart Association-Kansas Affiliate grant AHA-KS-98-GB-25, and a grant from the Dean’s Fund, College of Veterinary Medicine, Kansas State University, as well as a Research Career Enhancement Award from the American Physiological Society.
REFERENCES